

CLAIMS

1. Composition useful in gene therapy comprising stabilized particles of cationic transfection agent(s)/nucleic acid complexes, characterized in that it incorporates, in addition, at least one nonionic surface-active agent in a sufficient quantity to stabilize the size of the said particles at a size of less than or equal to 160 nm.

2. Composition according to claim 1, characterized in that the cationic transfection agent and the nucleic acid are present therein in a charge ratio of between 1 and 6.

3. Composition according to claim 1 or 2, characterized in that the cationic transfection agent and the nucleic acid are present therein in a charge ratio of less than 4.

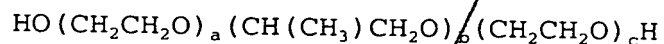
4. Composition according to one of the preceding claims, characterized in that the surface-active agent comprises at least one hydrophobic segment and at least one hydrophilic segment.

5. Composition according to claim 4, characterized in that the hydrophobic segment is chosen from aliphatic chains, polyoxyalkylenes, alkylidene polyesters, polyethylene glycols with a benzyl polyether head, and cholesterol.

6. Composition according to claim 4 or 5, characterized in that the hydrophilic segment is chosen

from polyoxyalkylenes, polyvinyl alcohols, polyvinylpyrrolidones, or saccharides.

7. Composition according to one of the preceding claims, characterized in that the surface-active agent is a polyoxyalkylene of general formula:



with a, b and c representing, independently of each other, integers which may vary between 20 and 100.

8. Composition according to one of the preceding claims, characterized in that it contains, as surface-active agent, a compound of general formula  $\text{OH}(\text{CH}_2\text{CH}_2\text{O})_a(\text{CH}(\text{CH}_3)\text{CH}_2\text{O})_b(\text{CH}_2\text{CH}_2\text{O})_c\text{H}$ , with a equal to 75, b to 30 and c to 75.

9. Composition according to one of the preceding claims, characterized in that it contains, as surface-active agent, a compound of the family of polyethylene glycol with a dendritic benzyl polyether head.

10. Composition according to one of the preceding claims, characterized in that it contains, as surface-active agent, a compound of the polyoxyethylene alcohol family.

11. Composition according to one of the preceding claims, characterized in that it contains, as surface-active agent, polyoxyethylene nonylphenyl ether.

12. Composition according to one of the preceding claims, characterized in that the surface-

active agent is present therein at a concentration of between 0.01% and 10% weight/volume of the said composition.

13. Composition according to one of the preceding claims, characterized in that the surface-active agent is present therein at a concentration of between 0.02% and 5% weight/volume of the said composition.

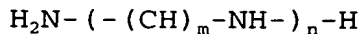
14. Composition according to one of the preceding claims, characterized in that the cationic transfection agent is a lipofectant.

15. Composition according to claim 14, characterized in that the lipofectant is an amphiphilic molecule comprising at least one lipophilic region combined or otherwise with a hydrophilic region.

16. Composition according to claim 14, characterized in that it is a lipid mixture capable of forming cationic liposomes.

17. Composition according to claim 14 or 15, characterized in that it is a cationic lipid.

18. Composition according to claim 14 or 15, characterized in that it is a lipofectant comprising at least one polyamine region of general formula:

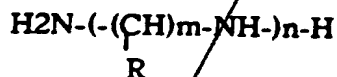


in which m is an integer greater than or equal to 2 and n is an integer greater than or equal to 1, it being possible for m to vary between the different groups of carbon between 2 amines, this polyamine region being

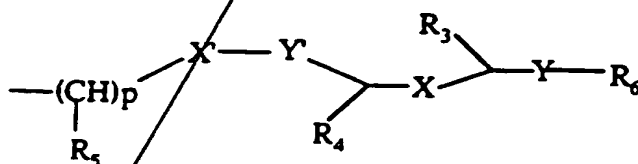
covalently combined with a lipophilic region of the saturated or unsaturated hydrocarbon chain of cholesterol type, or a natural or synthetic lipid capable of forming lamellar or hexagonal phases.

5                    19. Composition according to claim 18, characterized in that the polyamine region is represented by spermine or one of its analogues which has conserved its nucleic acid-binding properties.

10                    20. Composition according to claim 14 or 15, characterized in that it involves a lipofectant of general formula:

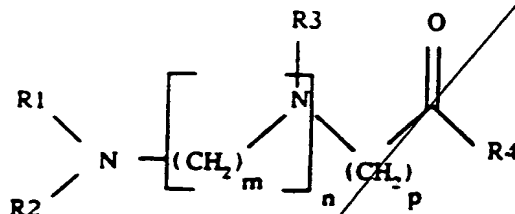


in which R denoting the lipophilic region is represented by the general formula:

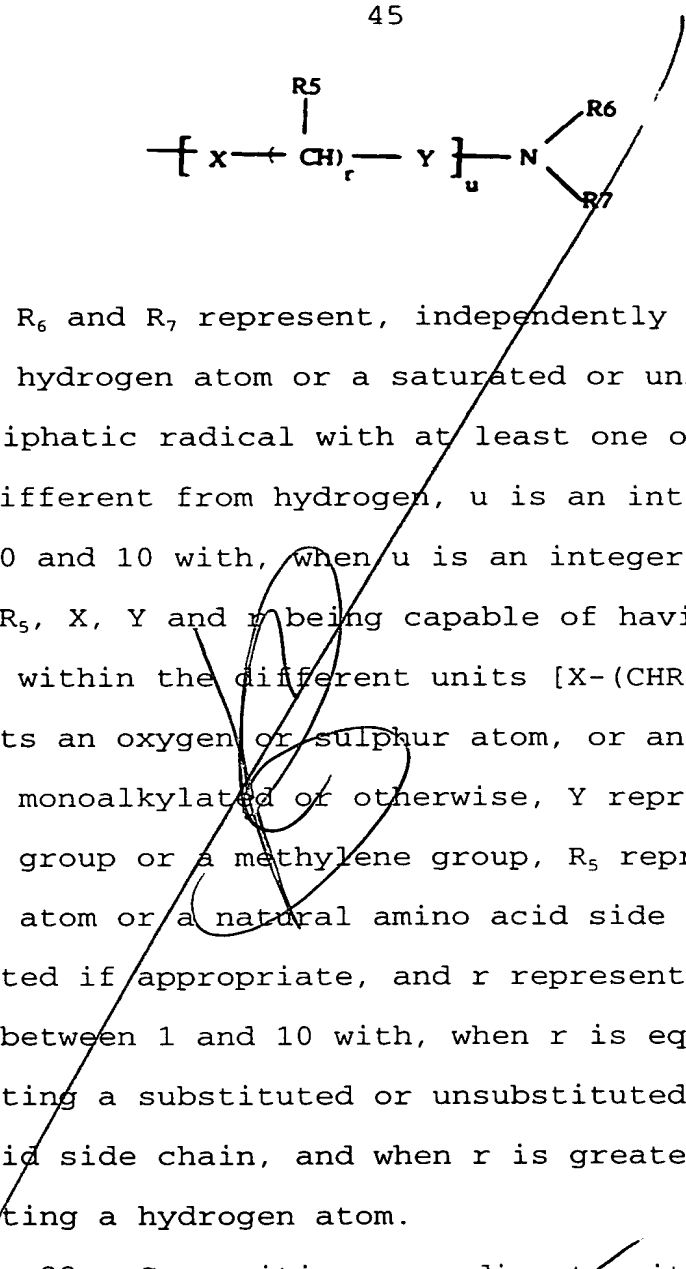


15                    in which X and X' represent, independently of each other, an oxygen atom, a methylene group  $-(\text{CH}_2)_q-$  with q equal to 0, 1, 2 or 3, or an amino group  $-\text{NH}-$  or  $-\text{NR}'-$ , with R' representing a  $\text{C}_1$  to  $\text{C}_4$  alkyl group, Y and Y' represent, independently of each other, a methylene group, a carbonyl group or a group  $\text{C}=\text{S}$ ,  $\text{R}_3$ ,  $\text{R}_4$  and  $\text{R}_5$  represent, independently of each other, a hydrogen atom  
20                    or a substituted or unsubstituted  $\text{C}_1$  to  $\text{C}_4$  alkyl

21. Composition according to claim 14 or 15,  
characterized in that it involves a lipofectant of  
general formula:



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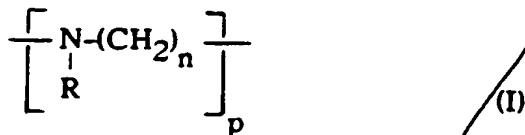
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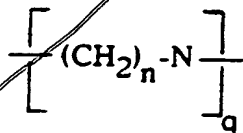
23. Composition according to one of claims 1 to 13, characterized in that the cationic transfection agent is a cationic polymer.

~~24. Composition according to claim 23,~~

characterized in that the said cationic polymer is a compound of general formula (I):



in which R may be a hydrogen atom or a group of formula:



5 n being an integer between 2 and 10, p and q being integers, it being understood that the sum p+q is such that the average molecular weight of the polymer is between 100 and 10<sup>7</sup> Da.

10 25. Composition according to claim 23 or 24, characterized in that it involves the polyethylene imine of average molecular weight 50,000 Da (PEI50K), the polyethylene imine of average molecular weight 22,000 Da (PEI22K) or the polyethylene imine of average molecular weight 800,000 Da (PEI800K).

15 26. Composition according to one of claims 1 to 14, characterized in that the cationic transfection agent is preferably chosen from lipofectamine, dioctadecylamidoglycyl spermine (DOGS), palmitoylphosphatidylethanolamine 5-carboxyspermylamide  
20 (DPPEs), 2,5-bis(3-aminopropylamino)pentyl

(dioctadecylcarbamoylethoxy) acetate,

5  $\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}(\text{CH}_2)_4\text{NHCH}_2\text{COGlyN}[(\text{CH}_2)_{17}\text{CH}_3]_2$ , and

$$\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}(\text{CH}_2)_3\text{NHCH}_2\text{COArgN}[(\text{CH}_2)_{17}\text{CH}_3]_2.$$

10                    28. Composition according to one of the  
preceding claims, characterized in that the nucleic  
acid is a ribonucleic acid.

29. Composition according to claim 27 or 28, characterized in that the nucleic acid is chemically modified.

30. Composition according to one of claims 1 to 26, characterized in that the nucleic acid is an antisense nucleic acid.

31. ~~Composition according to one of the preceding claims, characterized in that the nucleic acid comprises a therapeutic gene.~~

32. Composition according to one of the preceding claims, characterized in that it comprises, in addition, an adjuvant of the type comprising dioleoylphosphatidylethanolamine (DOPE), oleoylpalmitoylphosphatidylethanolamine (POPE), di-stearoyl, -palmitoyl, -myristoyl phosphatidyl-ethanolamines as well as their derivatives which are



N-methylated 1 to 3 times, phosphatidylglycerols, diacylglycerols, glycosyldiacylglycerols, cerebroside (such as in particular galactocerebrosides), sphingolipids (such as in particular sphingomyelins) or  
5 alternatively asialogangliosides.

33. Composition according to one of the preceding claims, characterized in that it combines, in addition, a targeting element with the cationic transfection agent.

10 34. Composition according to claim 33, characterized in that this targeting element is chosen from antibodies directed against molecules of the cellular surface, membrane receptor ligands such as insulin, transferrin, folic acid or any other growth  
15 factor, cytokines or vitamins, lectins, modified or otherwise, proteins with an RGD unit, peptides containing a tandem array of RGD units, cyclic or otherwise, polylysine peptides as well as natural or synthetic ligand peptides.

20 35. Process for the preparation of a composition comprising particles of cationic transfection agent(s)/nucleic acid complexes, characterized in that the transfecting agent and the nucleic acid are brought into contact in the presence  
25 of a sufficient quantity of a nonionic surface-active agent to stabilize the particles of nucleic complexes thus formed at a size of less than about 160 nm.

36. Process according to claim 35,

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37. Process according to claim 35 or 36, characterized in that the surface-active agent is defined therein according to claims 4 to 13.

add 4

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